Legends to Supplementary Figures

Figure S1. Lmp1 facilitates *B. burgdorferi* binding to CHO-K1 cells. Wild-type *B. burgdorferi* cells (WT), *lmp1* mutants (Δlmp1), and *lmp1*-complemented isolates (lmp1-com) were radiolabeled with [^{35}S] methionine and incubated with cultured CHO-K1 cells. After washing, radioactivity of the bound cells was measured using a liquid scintillation counter. The percentage of cell binding was determined by scintillation counting normalized to counts in the inoculum (see Experimental Procedures). Each bar represents the mean of 12 independent determinations ± SEM. As expected, the B314 isolate, which lacks multiple endogenous plasmids, displayed diminished binding activity that can be restored with overexpressed adhesins, like DbpA. Significant differences in Δlmp1 binding to the coated wells relative to the wild-type isolate were recorded (*, $P < 0.05$).

Figure S2. Plasmid profiling of *lmp1*-mutant isolate producing Lmp1M. PCR amplification with primers specific for each of the known endogenous *B. burgdorferi* plasmids was used to show the plasmid content of the isolate M-com. All of the endogenous parental plasmids are detectable. DNA size standards are shown in kilobase pairs (kbp).

Figure S3. Lmp1M mediates attachment of *B. burgdorferi* to various cell lines by binding to chondroitin-6-sulfate. **(A)** A variety of cell lines, representing epithelium (A549 and CHO-K1), synovial fibroblasts (SW982), endothelium (SVEC), and glia (C6), were incubated with wild type (WT), *lmp1* mutant (Δlmp1), or *lmp1* mutant producing full-length Lmp1 (lmp1-com) or middle region (M-com). Cells without *B. burgdorferi* was included as a negative control. Bound bacteria were stained with FITC-conjugated anti-*B. burgdorferi* antibody, and the percentage of cells bound to *B. burgdorferi* was measured by flow cytometry. The isolate Δlmp1 showed impaired binding capacity compared to WT, which can be restored with complementation of either full-length (lmp1-com) or middle region of *lmp1* (M-com). Each bar represents the mean of 12 independent determinations ± SEM. Significant differences in percentage of cells bound by each isolate of *B. burgdorferi* relative to wild type (WT) were determined by Student's *t*-test and are indicated (*, $P< 0.05$). **(B)** Chondroitin-6-sulfate competes with the binding of M-com to cells. Chondroitin-4-sulfate (Chon 4 SO_4) or

chondroitin-6-sulfate (Chon 6 SO_4) was added to the indicated *B. burgdorferi* isolates to a final concentration of 6.25 mg/ml prior to incubation with CHO-K1 cells in suspension (see Experimental Procedures). PBS alone ("None") was utilized as a negative control. The bacteria were stained with FITC-conjugated anti-*B. burgdorferi* antibody. The percentage of *B. burgdorferi*-bound cells was measured by flow cytometry. Each bar represents the mean of 8 independent determinations ± SEM. Statistically significant reductions in the percentage of cells bound by treated spirochetes compared to PBS-treated spirochetes are indicated (*, $P<0.05$).